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Synthesis of some S-3'-deoxyadenosyl-L-homocysteine analogues

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ABSTRACT

Condensation of 3'-deoxy-3-deazaadenosine, 3'-deoxy-7-deazaadenosine and 3'-deoxyadenosine with N,N'-bis-trifluoroacetyl-L-homocystine dimethyl ester and subsequent deprotection of the resulting N-trifluoroacetyl-S-3'-deoxyadenosyl-L-homocysteine analogues afforded S-3'-deoxy-3-deaza-adenosyl-L-homocysteine, S-3'-deoxy-7-deazaadenosyl-L-homocysteine and S-3'-deoxyadenosyl-L-homocysteine respectively. 3'-Deoxy-3-deazaadenosine and 3'-deoxy-7-deazaadenosine were prepared by transformation of the corresponding ribonucleosides with 2-acetoxyisobutyryl bromide. 3'-Deoxy-7-deazaadenosine and 3'-deoxyadenosine were also converted into their 5'-chloro-3',5'-dideoxy derivatives which in turn were condensed with L-homocysteine sodium salt to give S-3'-deoxy-7-deazaadenosyl-L-homo-cysteine and S-3'-deoxyadenosyl-L-homocysteine which were identical with those synthesised by condensation of the protected L-homocystine with the 3'-deoxynucleosides.

INTRODUCTION

S-Adenosyl-L-homocysteine and many of its analogues are potent inhibitors of biological methylation reactions catalysed by S-adenosyl-methionine dependent methyltransferases.[1] A large number of S-adenosyl-L-homocysteine congeners have been synthesised with modifications to the sugar moiety, amino acid residue or purine ring. In structure-activity studies these compounds have provided considerable information concerning the structural requirements for the binding of S-adenosyl-L-homocysteine derivatives to and inhibition of various specific methyltransferases.[2]

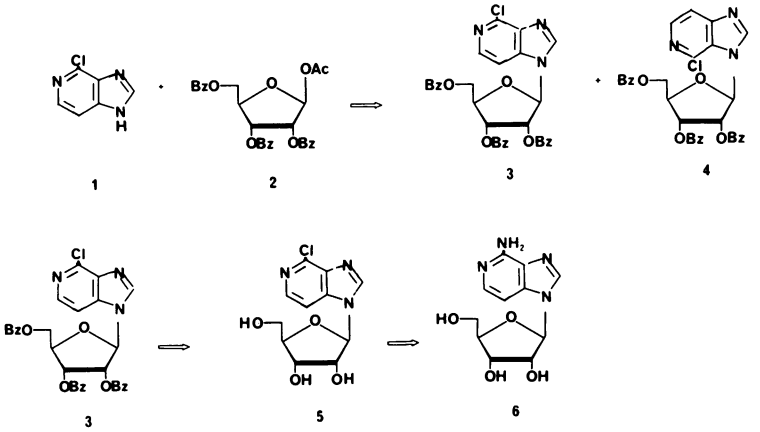
S-3-Deazaadenosyl-L-homocysteine and S-7-deazaadenosyl-L-homocysteine are two of the most potent inhibitors of certain methyltransferases described to date.[3] A new approach to the synthesis of these analogues which involves condensation of an unprotected nucleoside with N,N'-bis-trifluoroacetyl-L-homocystine dimethyl ester has been recently reported.[4] The use of other protecting groups for the amino and carboxyl functions of L-homocystine is under investigation and the results will be published

elsewhere.[5] Here, the synthesis of 3'-deoxy counterparts of S-3-deaza-adenosyl-L-homocysteine, S-7-deazaadenosyl-L-homocysteine and S-adenosyl-L-homocysteine is described. These compounds have been designed to investigate the effect of structural changes both in the heterocyclic ring and in the sugar moiety on their activities as methyltransferase inhibitors.

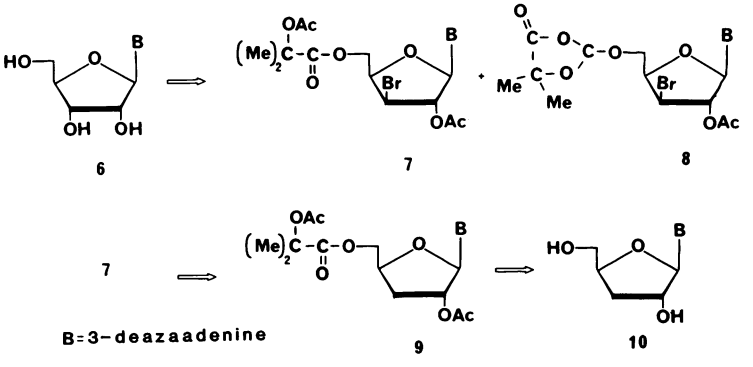
RESULTS AND DISCUSSION

The synthesis of S-3'-deoxy-3-deazaadenosyl-L-homocysteine, S-3'-deoxy-7-deazaadenosyl-L-homocysteine and S-3'-deoxyadenosyl-L-homocysteine involved preparation of the corresponding 3'-deoxy-nucleosides followed by introduction of the L-homocysteine residue into the 5'-position.

3'-Deoxy-3-deazaadenosine was prepared according to the route outlined in Schemes 1 and 2. The unsilylated 4-chloro-1H-imidazo[4,5-c]pyridine[6]



Scheme 1



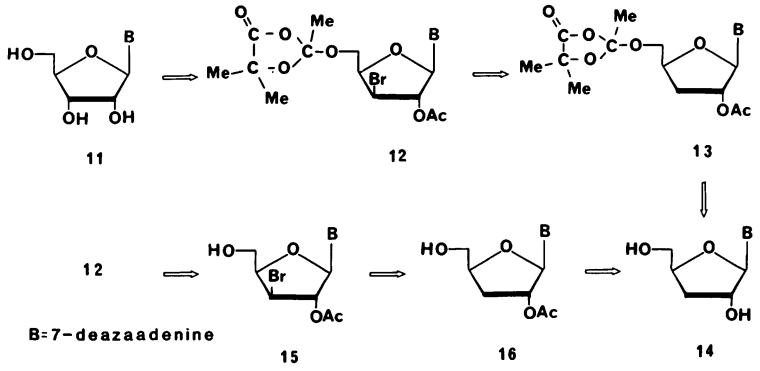
Scheme 2 B=3-deazaadenine

(1) was ribosylated with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (2) in acetonitrile in the presence of stannic chloride. The ribosylation showed high regioselectivity for the N-1 isomer (3) which was isolated in 72% yield whereas the product of ribosylation at N-3 (4) was formed in only 4% yield. Following debenzoylation of (3) successive reactions of the resulting 4-chloro-1-(β-D-ribofuranosyl)imidazo[4,5-c]pyridine (5) with anhydrous hydrazine and Raney Nickel afforded 3-deazaadenosine (6) in 85% yield based on (5). When 3-deazaadenosine (6) was allowed to react with 2-acetoxyisobutyryl bromide[7,9] in acetonitrile two major products were isolated by silica gel column chromatography. The structures of those compounds were established to be 4-amino-1-[3-bromo-3-deoxy-2-O-acetyl-5-O-(2-acetoxyisobutyryl)-β-D-xylofuranosyl]imidazo[4,5-c]pyridine (7) and 4-amino-1-[3-bromo-3-deoxy-2-O-acetyl-5-O-(2,5,5-trimethyl-4-oxo-1,3-dioxolan-2-yl)-β-D-xylofuranosyl]imidazo[4,5-c]pyridine (8) on the basis of ^{1}H NMR spectroscopy. The yields of (7) and (8) were calculated as 62 and 11% respectively. The ratio of both isomers (7) and (8) changed during the course of the reaction and after 48 h reached about 6:1 as the amount of (7) increased.

It is likely that 5'-O-dioxolano isomer (8) as a kinetically controlled product isomerised under acidic reaction conditions to give the more stable 5'-O-acetoxyisobutyrate (7). A preference for either dioxolano or acetoxyisobutyrate substitution at the 5'-position was observed previously by Moffat in the formycin, tubercidin and uridine series depending upon the heterocyclic base or solvent used for the reaction.[7,8,9]

Treatment of (7) with an excess of tri-n-butyltin hydride in the presence of 2,2'-azobis-(2-methylpropionitrile) in benzene afforded 4-amino-1-[3-deoxy-2-O-acetyl-5-O-(2-acetoxyisobutyryl)-β-D-ribofuranosyl]-imidazo[4,5-c]pyridine (9) in 83% yield. Finally 3'-deoxy-3-deaza-adenosine (10) was obtained after quantitative removal of 2' and 5'-O-protecting groups from (9) with methanolic ammonia. The ^{1}H NMR spectrum of (10) revealed the presence of signals at 2.17 and 1.93 ppm (δ scale) which were assigned to the geminal C-3' protons. A similar characteristic pattern of the chemical shift had been previously observed for C-3' hydrogens of cordycepin,[10,11,12] 3'-deoxyformycin[8] and other nucleosides. The structure of (10) was also confirmed by crystallographic analysis.[13]

3'-Deoxy-7-deazaadenosine (3'-deoxytubercidin) (14) was prepared in a similar way (Scheme 3). 4-Amino-7-[2-O-acetyl-3-bromo-3-deoxy-5-O-(2,5,5-



Scheme 3

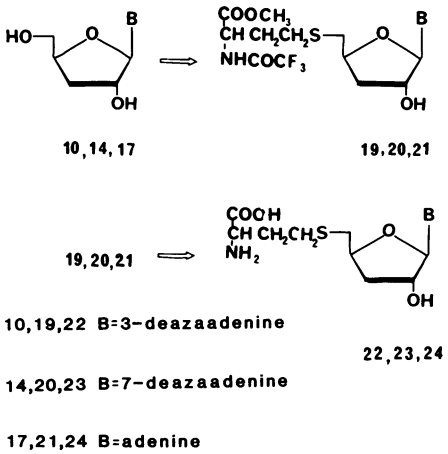
trimethyl-1,3-dioxolan-4-one-2-yl)-β-D-xylofuranosyl]pyrrollo[2,3-d]-pyrimidine (12) the sole product of the reaction of tubercidin (11) with 2-acetoxyisobutyryl bromide[8] was treated with tri-n-butyltin hydride in benzene in the presence of 2,2'-azobis-(2-methylpropionitrile) to give 4-amino-7-[2-O-acetyl-3-deoxy-5-O-(2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl)-β-D-ribofuranosyl]pyrrolo[2,3-d] pyrimidine (13). Deprotection of (13) with methanolic ammonia furnished 3'-deoxytubercidin (14) in 83% overall yield. 3'-deoxytubercidin was also obtained after selective removal of 5'-O-dioxolano group from (12) with 0.2 M methanolic hydrogen chloride, reduction of the resulting 4-amino-7-[2-O-acetyl-3-bromo-3-deoxy-(β-D-xylo-furanosyl)]pyrrolo[2,3-d]pyrimidine (15) with tri-n-butyltin hydride and deacetylation of the 2'-O-acetyl-3'-deoxytubercidin (16).

The structure of the nucleoside (14) was confirmed by crystallographic analysis[13] as well as by spectroscopic data which were in good agreement with those reported earlier.[8]

Cordycepin (3'-deoxyadenosine) (17) was synthesised according to the method described previously.[12]

Subsequently the 3'-deoxynucleosides (10), (14), (17) (Scheme 4) were condensed with N,N'-bis-trifluoroacetyl-L-homocystine dimethyl ester (18) in pyridine in the presence of tri-n-butylphosphine[4] to give the expected N-trifluoroacetyl-S-3'-deoxyadenosyl-L-homocysteine derivatives (19), (20) and (21) (Tables 1 and 2).

The yield of the condensation (Table 1) was highest for 3'-deoxy-3-deazaadenosine and lowest for 3'-deoxyadenosine. It is conceivable that in the case of the former which lacks N-3 nitrogen, the syn conformation in solution is not stabilised by a 5'-OH....N-3 hydrogen bond[14], and the



Scheme 4

attack of the bulky tri-n-butylphosphine-N,N'-bis-trifluoroacetyl-L-homocystine dimethyl ester complex[15] at the 5'-position is less sterically hindered than for nucleosides (14) and (17). A similar order of reactivity was observed for the ribo counterparts of (10), (14), and (17)[4].

Examination of the reaction mixtures by HPTLC revealed the presence of small amounts (5-10%) of undesired side products which probably derived from substitution at 2'-OH and 6-NH_2. Protection of those functions could possibly eliminate the formation of side products. Introduction of protecting groups, however, would involve extra steps and the overall yields would be substantially reduced. Attempts were made to increase the rate of the condensation by the use of triethylphosphine instead of tri-n-butylphosphine. Triethylphosphine is more reactive in the reductive cleavage of some disulphides.[16] In the case of N,N'-bis-trifluoroacetyl-L-homocystine dimethyl ester (18), however, the change had little or no effect as did the use of dimethylformamide instead of pyridine as a solvent. N-Trifluoroacetyl-S-3'-deoxyadenosyl-L-homocysteine derivatives (19), (20), and (21) could be readily purified by column chromatography on silica gel. The fact that the substitution had occurred at the 5'-position was confirmed by their ^1H NMR spectra which revealed the presence of signals at about 5.5 ppm (δ scale) assigned to the 2'-OH groups of the sugar moiety.

The protecting groups were removed from (19), (20), and (21) with 0.25M barium hydroxide[4] during 2 h at room temperature to give S-3'-deoxy-

Table 1: S-3'-deoxyadenosyl-L-homocysteine derivatives and 5'-chloro-3',5'-dideoxynucleosides prepared

Product No	Reaction time /h/	yield %	M.p. °C	M.p. lit. °C	Molecular Formula	Requires:	Found:
19	120	58	indef.	-	$C_{18}H_{22}F_3N_5O_5S$	C, 45.28; H, 4.65; N, 14.67	C, 45.54; H, 5.23; N, 14.50%
20	96	46	indef.	-	$C_{18}H_{22}F_3N_5O_5S$	C, 45.28; H, 4.65; N, 14.67	C, 44.96; H, 4.85; N, 14.45%
21	72	37	indef.	-	$C_{17}H_{21}F_3N_6O_5S.H_2O$	C, 41.12; H, 4.66; N, 16.92	C, 40.58; H, 4.66; N, 16.78%
22	2	92	indef.	-	$C_{15}H_{21}N_5O_4S.2H_2O$	C, 44.65; H, 6.25; N, 17.35	C, 44.39; H, 5.42; N, 17.16%
23	2	87	>145 indef. H_2O/EtOH	-	$C_{15}H_{21}N_5O_4.1.5H_2O$	C, 45.67; H, 6.13; N, 17.75	C, 45.52; H, 6.14; N, 17.57%
24	2	90	211-213 H_2O/EtOH	211[18]	$C_{14}H_{20}N_6O_4S.2H_2O$	C, 41.57; H, 5.98; N, 20.77	C, 41.02; H, 5.25; N, 20.45%
25	16	81	indef. H_2O	-	$C_{11}H_{13}ClN_4O_2.H_2O$	C, 46.08; H, 5.27; N, 19.54	C, 46.61; H, 5.09; N, 19.85%
26	16	83	indef. H_2O	-[18]	$C_{10}H_{12}ClN_5O_2.0.75H_2O$	C, 42.41; H, 4.80; N, 24.73	C, 42.46; H, 4.78; N, 24.98%

3-deazaadenosyl-L-homocysteine (22), S-3'-deoxy-7-deazaadenosyl-L-homocysteine (23) and S-3'-deoxyadenosyl-L-homocysteine (24) in high yields (Table 1). After purification on Sephadex A-25 the compounds were homogeneous on HPLC and gave positive tests with ninhydrin.

3'-Deoxytubercidin (14) and 3'-deoxyadenosine (17) were also converted into their 5'-chloro-3',5'-dideoxyderivatives (25) and (26) by treatment with thionyl chloride in trimethyl phosphate[17] (Tables 1 and 2) (Scheme 5). Compounds (25) and (26) were subsequently condensed with L-homocysteine sodium salt in liquid ammonia. The resulting S-3'-deoxy-7-deazaadenosyl-L-homocysteine (23) and S-3'-deoxyadenosyl-L-homocysteine (24) were purified on Sephadex A-25 and were identical with those prepared by condensation of the protected L-homocystine with unprotected nucleosides.

The compounds are undergoing biochemical evaluation and the results will be published elsewhere.

EXPERIMENTAL

^{1}H Nmr spectra were recorded at 250 MHz with a Bruker WH 250 spectrometer. Tetramethylsilane was used as an internal standard and DMSO-d_6 as a solvent unless otherwise indicated. UV spectra were measured in 95% ethanol with a Pye-Unicam SP8-150 UV-Vis spectrometer. Merck silica gel 60F_{254} [developed with chloroform-ethanol (19:1) system A, chloroform-ethanol (9:1) system B, chloroform-ethanol (4:1) system C and benzene-ethyl acetate (4:1) system D] and Merck DC-Alufolien Cellulose F sheets [developed in n-butanol-acetic acid-water (12:3:5) system E] were used for tlc. Column chromatography was carried out on silica gel 60 (230-400 mesh) (Merck) and short column chromatography on silica gel 60H (Merck). Pyridine and acetonitrile were dried by heating under reflux with calcium hydride and subsequent distillation. The solvents were stored over molecular sieves 4Å and 3Å respectively. Tubercidin and L-homocystine were purchased from SIGMA.

4-Chloro-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazo[4,5-c]pyridine (3) and 4-chloro-3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazo-[4,5-c]pyridine(4). 4-Chloro-1H-imidazo[4,5-c]pyridine[6] (1) (1.078 g, 7 mmol) and 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (2) (3.78 g, 7.5 mmol) were suspended in acetonitrile (100 ml). To the stirred suspension a solution of stannic chloride (1.5 ml, 12.78 mmol) in acetonitrile (50 ml) was added. After 30 min the solution became clear and the stirring was continued for 6 h at room temperature. Then about half of the solvent was evaporated under reduced pressure, chloroform (150 ml) was

added and the solution poured onto 5% aqueous sodium hydrogen carbonate (250 ml). The organic layer was separated and the aqueous phase was further extracted with chloroform (6 x 30 ml). The chloroform extracts

Table 2: UV and NMR spectra of S-3'-deoxyadenosyl-L-homocysteine derivatives and 5'-chloro-3',5'-dideoxynucleosides

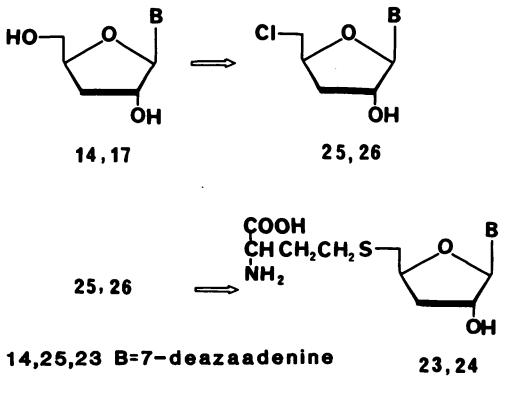
Product No.	UV	^{1}H NMR (ppm, δ scale)[a]
19	λ_{max} 268 nm (ϵ,10960) λ_{min} 235 nm (ϵ,2430)	1.95-2.15 (4Hm, H-3', H-3'', H-β) 2.60 (2H, m, H-γ) 2.85 (2H, m, H-5', H-5'',) 3.65 (3H, s, OCH_3) 4.47 (3H, m, H-2', H-4', H-α) 5.77 (1H, d, H-1') 6.31 (2H, bs, NH_2) 6.86 (1H, d, H-3), 7.69 (1H, d, H-2) 8.21 (1H, s, H-8) 9.85 (1H, d, $NHCOCF_3$)
20	λ_{max} 270 nm (ϵ,26080) λ_{min} 240 nm (ϵ,7130)	1.96-2.17 (4H, m, H-3', H-3'', H-β) 2.60 (2H, m, H-γ) 2.82 (2H, m, H-5', H-5'') 3.65 (3H, s, OCH_3,) 4.43 (3H, m, H-2', H-4', H-α) 5.59 (1H, d, OH-2') 6.05 (1H, d, H-1') 6.60 (1H, d, H-7) 6.99 (2H, bs, NH_2) 7.23 (1H, d, H-8) 8.06 (1H, s, H-2) 9.85 (1H, d, $NHCOCF_3$)
21	λ_{max} 260 nm (ϵ,12770) λ_{min} 228 nm (ϵ,2500)	1.98-2.28 (4H, m, H-3', H-3'', H-β) 2.60 (2H, m, H-γ) 2.84 (2H, m, H-5', H-5'') 3.61 (3H, s, OCH_3) 4.45 (2H, m, H-4', H-α) 4.66 (1H, m, H-2') 5.70 (1H, d, OH-2') 5.88 (1H, d, H-1') 7.27 (2H, bs, NH_2) 8.14 (1H, s, H-2) 8.24 (1H, s, H-8) 9.83 (1H, bs, $NHCOCF_3$)
22	λ_{max} 270 nm (ϵ,13790) λ_{min} 235 nm (ϵ,5340)	1.86-2.00 (4H, m, H-3', H-3'', H-β) 2.66 (2H, t, H-γ) 2.84 (2H, m, H-5', H-5'') 3.33 (1H, m, H-α) 4.03 (1H, m, H-4') 4.42 (1H, m, H-2') 5.76 (1H, d, H-1') 6.21 (2H, bs, NH_2), 6.82 (1H, d, H-3) 7.68 (1H, d, H-2) 8.27 (1H, s, H-8)
23 NMR in D_2O	λ_{max} 271 nm (ϵ,9040) λ_{min} 240 nm (ϵ,1150)	1.98-2.28 (4H, m, H-3', H-3', H-β), 2.64 (2H, t, H-γ), 2.85 (2H, m, H-5', H-5''), 3.71 (IH, t, H-α) 4.65 (2H, m, H-2', H-4'), 6.12 (1H, d, H-1'), 6.54 (1H, d, H-7), 7.22 (1H, d, H-8), 8.05 (1H, s, H-2)
24	λ_{max} 259 nm (ϵ,7370)	1.85-2.30 (4H, m, H-3', H-3'', H-β) 2.65 (2H, t, H-γ) 2.85 (2H, m, H-5', H-5''), 4.01 (1H, t, H-α) 4.43 (1H, m, H-4') 4.65 (1H, m, H-2') 5.89 (1H, d, H-1') 7.30 (2H, bs, NH_2 8.15 (1H, s, H-2) 8.27 (1H, s, H-8)

Table 2 (Continued)

Product No.	UV	^{1}H NMR (ppm, δ scale)[a]
25	λ_{max} 270 nm (ϵ,9930) λ_{min} 240 nm (ϵ,2970)	2.06 (1H, m, H-3′) 2.25 (1H, m, H-3″) 3.82 (2H, m, H-5′, H-5″), 4.48 (2H, m, H-2′, H-4′) 5.66 (1H, d, OH-2′) 6.09 (1H, d, H-1′) 6.61 (1H, d, H-7) 7.03 (2H, bs, NH_2) 7.25 (1H, d, H-8) 8.07 (1H, s, H-2)
26	λ_{max} 260 nm (ϵ, 12900) λ_{min} 227 nm (ϵ, 1030)	2.11 (1H, m, H-3′) 2.36 (1H, m, H-3″) 3.90 (2H, m, H-5′, H-5″) 4.52 (1H, m, H-4′) 4.73 (1H, m, H-2′) 5.77 (1H, d, OH-2′) 5.92 (1H, d, H-1′) 7.29 (2H, bs, NH_2) 8.16 (1H, s, H-2) 8.26 (1H, s, H-8)

[a] 2′-OH, 3′-OH, NH_2 and $NHCOCF_3$ protons were exchangeable with D_2O.

were combined, dried over anhydrous sodium sulphate and evaporated to give a white foam which was dissolved in small amount of benzene and applied to a silica gel column. Elution of the column with benzene and subsequently with benzene-ethyl acetate (4:1) afforded the product (4) (0.15 g, 4%) as a white froth which after recrystallisation from methanol gave colourless needles m.p.98-101°C, (Found: C, 64.15; H, 4.02; N, 7.11. $C_{32}H_{24}ClN_3O_7$ requires C, 64.27; H, 4.06; N, 7.03%); nmr δ 4.81 (2H, m, H-5′, H-5″), 4.95 (1H, m, H-4′), 6.04 (1H, m, H-3′), 6.23 (1H, m, H-2′), 7.12 (1H, d, H-1′), 7.40-8.05 (16H, m, phenyls and H-7) 8.24 (1H, d, H-6), 8.98 (1H, s, H-2). Further elution of the column with benzene-ethyl acetate (4:1)



Scheme 5

yielded the product (3) isolated as a white foam (3.05 g, 72.8%). After crystallisation from methanol it had m.p.108-110°C (lit.[19] 110-111°C); (Found: C, 64.04; H, 4.06; N, 6.92. $C_{32}H_{24}ClN_3O_7$ requires: C, 64.27; H, 4.06; N, 7.03%); nmr δ 4.82 (2H, m, H-5', H-5''), 4.92 (1H, m, H-4'), 6.04 (1H, m, H-3'), 6.12 (1H, m, H-2'), 6.78 (1H, d, H-1'), 8.10-7.35 (17H, m, phenyls, H-6 and H-7), 8.85 (1H, s, H-2).

4-Chloro-1-(β-D-ribofuranosyl)imidazo[4,5-c]pyridine(5). 4-Chloro-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazo[4,5-c]pyridine (3) and 8M methanolic ammonia (100 ml) were stirred together at room temperature for 12h. The solvent was removed under reduced pressure and the residue dissolved in water (250 ml). The aqueous solution was extracted with chloroform (4 x 25 ml), ether (1 x 20 ml) and then was concentrated to dryness to give 1.56g (98%) of (5) as a white powder which after crystallisation from ethanol afforded colourless needles, m.p. 195-197°C (lit.[19] 200-201°C);(Found: C,46.17; H, 4.30; N, 14.35. $C_{11}H_{12}ClN_3O_4$ requires: C, 46.25; H, 4.23; N, 14.71%); nmr δ 3.66 (2H, m, H-5', H-5''), 4.01 (1H, m, H-4'), 4.13 (1H, m, H-3'), 4.35 (1H, m, H-2'), 5.22 (1H, t, 5'-OH,), 5.33 (1H, d, 3'-OH), 5.59 (1H, d, 2' -OH), 5.93 (1H, d, H-1'), 7.92 (1H, d, H-7), 8.71 (1H, s, H-2).

4-Amino-1-(β-D-ribofuranosyl)imidazo[4,5-c]pyridine (3-deazaadenosine) (6). 4-Chloro-1-(β-D-ribofuranosyl)imidazo[4,5-c]pyridine (5) (2.20g, 7.72 mmol) was dissolved in anhydrous hydrazine (30 ml) and the solution was heated under reflux with stirring and exclusion of moisture for 80 min. The hydrazine was removed in vacuo to afford an amber residue which was co-evaporated with ethanol (2 x 50 ml). The residue was dissolved in 50% aqueous ethanol (200 ml), Raney-Nickel catalyst (10 g) was added and the mixture refluxed for 2 h with stirring. The catalyst was filtered off, washed with 50% aqueous ethanol and the combined solutions were concentrated to give 1.73 g of (6). Crystallisation from 95% aqueous isopropanol afforded white crystals, m.p. 226-228°C, (lit.[20-22] 229-231°C); uv λ_{max} 267 nm (ε,10070), λ_{min} 231 nm (ε,3010). (Found: C, 49.29; H, 5.34; N, 21.42. $C_{11}H_{14}N_4O_4$ requires C, 49.62; H, 5.30; N, 21.04%); nmr δ 3.65 (2H, m, H-5', H-5''), 4.01 (1H, m, H-4'), 4.13 (1H, m, H-3'), 4.30 (1H, m, H-2'), 5.24 (1H, bs, OH-2'), 5.33 (1H, bs, OH-3'), 5.63 (1H, bs, OH-5'), 5.89 (1H, d, H-1'), 7.30 (1H, d, H-3), 7.73 (1H, d, H-2), 7.94 (2H, bs, NH_2), 8.59 (1H, s, H-8).

4-Amino-1-[2-O-acetyl-3-bromo-3-deoxy-5-O-(2-acetoxyisobutyryl)-β-D-xylofuranosyl]imidazo[4,5-c]pyridine (7) and 4-amino-1-[2-O-acetyl-3-bromo-3-deoxy-5-O-(2,5,5-trimethyl-4-oxo-1,3-dioxolan-2-yl)-β-D-xylofuranosyl] imidazo[4,5-c]pyridine (8). A suspension of 3-deazaadenosine (6) (0.79 g, 3 mmol) in 2-acetoxyisobutyryl bromide[7,9] (2.82g, 13.5 mmol) and acetonitrile (30 ml) was stirred at room temperature for 48 h. The resulting clear pale green-yellow solution was evaporated in vacuo and the residue partitioned between ethyl acetate (50 ml) and 5% aqueous sodium hydrogen carbonate (50 ml). The organic and aqueous layers were separated and the latter extracted with ethyl acetate (4 x 25 ml). The extracts were combined, dried over anhydrous sodium sulphate and evaporated in vacuo to give a white froth (1.42 g). The crude product was applied to a silica gel column which was eluted with chloroform-ethanol (95:5) to give 0.93 g (62%) of (7) as a white foam. The foam was dissolved in a small amount of chloroform and added dropwise to a stirred light petroleum (b.p.30-40°C) (300 ml). The resulting white precipitate was collected by centrifugation and dried in a desiccator, uv λ_{max} 267 nm (ϵ, 9270), λ_{min} 237 nm (ϵ, 2750); (Found: C, 45.55; H, 4.66; N, 11.09 $C_{19}H_{23}BrN_4O_7$ requires C, 45.70; H, 4.64; N, 11.22%); nmr δ ($CDCl_3$) 1.58 (6H, s, $(CH_3)_2C$), 2.06 (3H, s, $OCOCH_3$, 2.21 (3H, s, $OCOCH_3$), 4.47 (2H, m, H-5', H-5''), 4.55 (2H, m, H-2', H-4'), 5.61 (1H, m, H-3'), 5.76 (2H, bs, NH_2), 5.90 (1H, d, H-1'), 6.85 (1H, d, H-3), 7.82 (1H, d, H-2), 8.24 (1H, s, H-8). Further elution of the column with chloroform-ethanol (19:1) afforded 0.17 g (11%) of (8) as a white foam which was dissolved in a small amount of chloroform and added dropwise to a stirred light petroleum (b.p. 30-40°C) (50 ml) to give a white precipitate which was collected by centrifugation and dried in a desiccator, uv λ_{max} 268 nm (ϵ, 18740), λ_{min} 240 nm (ϵ,7370); (Found: C, 45.67; H, 4.71; N, 11.43, $C_{19}H_{23}BrN_4O_7$ requires C, 45.70; H, 4.64; N, 11.43%); nmr δ ($CDCl_3$) 1.59 (3H, s, CH_3C), 1.61 (3H, s, CH_3C) 2.06 (3H, s, CH_3-CO_3), 2.20 (3H, s, $OCOCH_3$), 4.38 (1H, m, H-2'), 4.57 (3H, m, H-5', H-5'', H-4'), 5.49 (1H, m, H-3'), 6.15 (1H, d, H-1'), 6.68 (1H, d, H-3), 7.87 (1H, d, H-2), 8.17 (1H, s, H-8).

4-Amino-1-[2-O-acetyl-3-deoxy-5-O-(2-acetoxyisobutyryl)-β-D-ribofuranosyl]imidazo[4,5-c]pyridine (9). 4-Amino-1-[2-O-acetyl-3-bromo-3-deoxy-5-O-(2-acetoxyisobutyryl)-β-D-xylofuranosyl]imidazo[4,5-c]pyridine (8) (0.99 g, 2 mmol) was dissolved in benzene (20 ml) and tri-n-butyltin hydride (2.37 g, 8.1 mmol) and 2,2'-azobis-(2-methylpropionitrile) (0.05 g, 0.3 mmol) were added. The reactants were heated under reflux with stirring

for 75 min and after cooling the mixture was added dropwise to light petroleum (b.p. 30-40°C) (250 ml). The resulting white precipitate was collected by filtration and then further washed with light petroleum. The crude product was applied to a silica gel column. The product (9) was eluted with chloroform-ethanol (93:7) to give 0.70 g (83%) of (9) as a white foam. An analytically pure sample was obtained by precipitation as described above for (7) and (8), uv λ_{max} 268 nm (ϵ , 10450), λ_{min} 235 nm (ϵ, 2130). (Found: C, 53.27; H, 5.92; N, 12.94. $C_{19}H_{24}N_4O_7.0.5H_2O$ requires: C, 53.14; H, 5.87; N, 13.05%); nmr δ 1.48 (6H, s, $(CH_3)_2$), 1.98 (3H, s, $OCOCH_3$), 2.08 (3H, s, $OCOCH_3$), 2.22 (1H, m, H-3'), 2.36 (1H, m, H-3''), 4.30 (2H, m, H-5', H-5''), 4.53 (1H, m, H-4'), 5.40 (1H, m, H-2'), 6.11 (1H, d, H-1'), 6.23 (2H, bs, NH_2), 6.88 (1H, d, H-3), 7.69 (1H, d, H-2), 8.21 (1H, s, H-8).

4-Amino-1-(3-deoxy-β-D-ribofuranosyl)imidazo[4,5-c]pyridine (3'-Deoxy-3-deazaadenosine) (10). 4-Amino-1-[2-O-acetyl-3-deoxy-5-O-(2-acetoxy-isobutyryl)-β-D-ribofuranosyl]imidazo[4,5-c]pyridine (9) (0.42g, 1 mmol) and 8 M methanolic ammonia (12 ml) were stirred together at room temperature for 48 h, then the reaction solution was stored at 0-4°C for 16 h. The resulting crystalline precipitate of (10) was collected by filtration. A second crop of (10) was obtained by recrystallising the evaporated mother liquors from absolute methanol ; total yield 0.23 g (91%), m.p. 225-226°C; uv λ_{max} 268 nm (ϵ , 10930), λ_{min} 233 nm (ϵ , 1920); Found: C, 52.92; H, 5.65; N, 22.62. $C_{11}H_{14}N_4O_3$ requires: C, 52.79; H, 5.64; N, 22.38%); nmr δ 1.93 (1H, m, H-3'), 2.27 (1H, m, H-3''), 3.57 (1H, m, H-5'), 3.69 (1H, m, H-5''), 4.39 (2H, m, H-2', H-4'), 5.07 (1H, bs, OH-2'), 5.75 (1H, d, H-1'), 6.20 (2H, bs, NH_2), 6.88 (1H, d, H-3), 7.68 (1H, d, H-2), 8.34 (1H, s, H-8).

4-Amino-7-[2-O-acetyl-3-deoxy-5-O-(2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl)-β-D-ribofuranosyl]pyrrolo[2,3-d]pyrimidine) (12). 4-Amino-7-[2-O-acetyl-3-bromo-3-deoxy-5-O-(2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl)-β-D-xylo-furanosyl]pyrrolo[2,3-d]pyrimidine[8] (11) (1.50 g, 3 mmol) was dissolved in benzene (30 ml) under an atmosphere of argon and tri-n-butyltin hydride (3.55 g, 12.15 mmol) and 2,2'-azobis-(2-methylpropionitrile) (0.08 g, 0.45 mmol) were added. The stirred reactants were heated under reflux. After 75 min the mixture was cooled and added dropwise to a stirred light petroleum (b.p. 30-40°C). The resulting white precipitate was collected by filtration and then further washed with petroleum ether. The crude product was applied to a column of silica gel, which was eluted with chloroform-

ethanol (94:6) to give 1.08 g (86%) of (12) as a white froth. An analytically pure sample was obtained when (12) was dissolved in a small amount of chloroform and added dropwise to a stirred light petroleum to give a white precipitate which was collected by centrifugation and dried in a desiccator, uv λ_{max} 270 nm (ϵ , 13500); λ_{min} 238 nm (ϵ , 3260) (Found: C, 53.53; H, 5.81; N, 13.25. $C_{17}H_{24}N_4O_7$ requires: C, 54.28; H, 5.75; N, 13.32%); nmr δ 1.44 (6H, s, $(CH_3)_2C$), 1.69 (3H, s, CH_3-CO_3), 1.98 (1H, m, H-3'), 2.07 (3H, s, $OCOCH_3$) 2.18 (1H, m, H-3''), 3.71 (2H, m, H-5', H-5''), 4.39 (1H, m, H-4'), 5.45 (1H, m, H-2'), 6.21 (1H, d, H-1'), 6.60 (1H, d, H-7), 7.07 (2H, bs, NH_2), 7.26 (1H, d, H-6), 8.05 (1H, s, H-2).

3'-Deoxytubercidin (3'-deoxy-7-deazaadenosine) (14). 4-Amino-7-[2-O-acetyl-3-deoxy-5-O-(2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl)-β-D-ribofuranosyl]-pyrrolo[2,3-d]pyrimidine (13) (1.26 g, 3 mmol) and 8 M methanolic ammonia (30 ml) were stirred together at room temperature. After 4 h the solvent was removed in vacuo and the residue dissolved in water (150 ml). The water solution was extracted with chloroform (4 x 25 ml) and diethyl ether (1 x 30 ml). The organic extracts were discarded and the aqueous solution evaporated under reduced pressure to give 0.71 g (95%) of colourless glassy residue. The crude product was crystallised from ethyl acetate to give analytically pure 3'-deoxytubercidin (14), m.p.178-180°C, (lit.[8] 178-179°C); uv λ_{max} 271 nm (ϵ , 10280), λ_{min} 241 nm (ϵ , 2964)(Found: C, 52.62; H, 5.60; N, 22.61. $C_{11}H_{14}N_4O_3$ requires: C, 52.79; H, 5.64; N, 22.39%); nmr δ 1.96 (1H, m, H-3'), 2.18 (1H, m, H-3''), 3.51, (1H, m, H-5'), 3.60 (1H, m, H-5'') 4.27 (1H, m, H-4'), 4.40 (1H, m, H-2'), 5.09 (1H, t, OH-5'), 5.54 (1H, bs, OH-2'), 6.01 (1H, d, H-1'), 6.56 (1H, d, H-7), 7.03 (2H, bs, NH_2), 7.33 (1H, d, H-8), 8.06 (1H, s, H-2).

4-Amino-7-[2-O-acetyl-3-bromo-3-deoxy-(β-D-xylofuranosyl)]pyrrolo[2,3-d]pyrimidine (15). A solution of (12) (0.50 g, 1 mmol) in 0.2 M methanolic hydrogen chloride (25 ml) was stirred at room temperature for 30 min. After addition of pyridine (1.5 ml) the solution was concentrated in vacuo and the residue dissolved in the mixture of 5% aqueous sodium hydrogen carbonate-ethyl acetate (1:1) (50 ml). The aqueous and organic layers were separated and the aqueous layer was extracted with ethyl acetate (3 x 10 ml). The extracts were combined, dried over anhydrous sodium sulphate and evaporated in vacuo. The residue was applied to a column of silica gel and the product was eluted with chloroform ethanol (85:15) to give (15), (0.305g, 82%) as a white foam. An analytically pure sample was prepared by precipitation to light petroleum ether as described for (12),

uv λ_{max} 269 nm (ϵ , 10960); λ_{min} 240 nm (ϵ , 3470). (Found: C, 39.50; H, 4.25; N, 13.55, $C_{13}H_{15}BrN_4O_4.1.5H_2O$ requires: C, 39.21; H, 4.55; N, 14.07%), nmr δ 2.07 (3H, s, $OCOCH_3$), 3.68 (2H, m, H-5', H-5''), 4.19 (1H, m, H-4'), 4.81 (1H, m, H-2'), 5.28 (1H, m, OH-5'), 5.62 (1H, m, H-3'), 6.26 (1H, d, H-1') 6.67 (1H, d, H-7), 7.13 (2H, bs, NH_2) 7.38 (1H, d, H-8), 8.07 (1H, d, H-2).

4-Amino-7-[2-O-acetyl-3-deoxy-(β-D-ribofuranosyl)]pyrrolo[2,3-d]pyrimidine (16). 4-Amino-7-[2-O-acetyl-3-bromo-3-deoxy-(β-D-xylofuranosyl)]pyrrolo-[2,3-d]-pyrimidine (15) (0.37g) (1 mmol) was suspended in benzene (10 ml) under an atmosphere of argon and tri-n-butyltin hydride (1.18g, 4.05 mmol) and 2,2'-azobis-(2-methylpropionitrile) (0.03 g, 0.15 mmol) were added. The stirred reactants were heated under reflux for 75 min. The solvent was removed under reduced pressure and the residue applied to a short column of silica gel which was eluted with chloroform-ethanol (88:12) to give the product (16) as a white foam (0.23 g, 79%). Precipitation as described for (12) afforded an analytically pure sample, uv λ_{max} 270 nm (ϵ , 11590), λ_{min} 239 nm (ϵ , 2910), (Found: C, 52.86; H, 5.40; N, 19.00 $C_{13}H_{16}N_4O_4$ requires: C, 53.42; H, 5.52; N, 19.16%), nmr δ 2.06 (3H, s, $OCOCH_3$), 2.10 (1H, m, H-3'), 2.45 (1H, m, H3''), 3.53 (1H, m, H-5'), 3.61 (1H, m, H-5''), 4.27 (1H, m, H-4'), 5.10 (1H, t, OH-5'), 5.41 (1H, m, H-2'), 6.18 (1H, d, H-1'), 6.59 (1H, d, H-7), 7.05 (2H, bs, NH_2), 7.34 (1H, d, H-8), 8.05 (1H, s, H-2).

3'-Deoxytubercidin (14). Compound (16) (0.29 g, 1 mmol) and 8 M methanolic ammonia (10 ml) were stirred together at room temperature for 4 h. The solvent was removed in vacuo and the residue dissolved in water (50 ml). The water solution was extracted with chloroform (3 x 10 ml) and diethyl ether (1 x 10 ml). The organic extracts were discarded and the aqueous layer was evaporated under reduced pressure to give 0.23 g (93%) of 3'-deoxytubercidin (14) as a colourless glass which after recrystallisation from ethyl acetate was identical with that obtained by deprotection of (13).

3'-Deoxyadenosine (cordycepin) (17). 3'-Deoxyadenosine was prepared according to the method described by Norman and Reese[12] m.p. 230-231°C (lit.[12] 221-224°C).

Condensation of 3'-deoxynucleosides (10), (14) or (17) with N,N'-bis-trifluoroacetyl-L-homocystine dimethyl ester (18). General procedure. A nucleoside (10), (14) or (17) (1 mmol) and N,N'-bis-trifluoroacetyl-L-homocystine dimethyl ester[4] (18) (3 mmol) were dissolved in dry pyridine

(10 ml) and the solvent was removed under reduced pressure. The procedure was repeated three times and the residue was dissolved in pyridine (5 ml) under argon and tri-n-butylphosphine (1.5 ml, 6 mmol) was added. The mixture was stirred at room temperature and the reaction was monitored by HPTLC (systems B and C). After 72-120 h methanol (10 ml) was added, the stirring was continued for further 20 min and the solvents were evaporated in vacuo. The resulting oily residue was coevaporated with pyridine (3 x 10 ml) and toluene (3 x 10 ml) and was applied to a short column of silica gel which was eluted with chloroform-ethanol (9:1) for (19), (19:1) for (20) and (93:7) for (21). The appropriate fractions were combined and evaporated to give (19), (20) and (21) as colourless oils. Each colourless oil was dissolved in small amount of chloroform with the addition of few drops of pyridine and was added dropwise to a stirred light petroleum (b.p. 30-40°C). The resulting white precipitate was collected by centrifugation and dried in a desiccator. (Tables 1 and 2).

Deprotection of N-trifluoroacetyl-S-3'-deoxyadenosyl-L-homocysteine derivatives (19), (20) and (21). General procedure. N-trifluoroacetyl-S-3'-deoxyadenosyl-L-homocysteine derivative (19), (20) or (21) (0.5 mmol) was dissolved in 0.25 M barium hydroxide in aqueous methanol (1:1), (10 ml). The solution was stirred at room temperature for 2 h, then neutralised with 1.0 M sulphuric acid and centrifuged. The supernatant was concentrated and applied to a column of Sephadex A-25 (2.5 x 20 cm) which was eluted with 0.01 M triethylammonium hydrogen carbonate buffer. The fractions containing the products (uv absorbing, ninhydrin positive) were combined, concentrated and lyophilised to give (22), (23) and (24) respectively. (Tables 1 and 2).

5'-Chloro-3',5'-dideoxynucleosides (25) and (26). General procedure. 3'-Deoxynucleoside (14) or (17) (1 mmol) was added to a stirred solution of thionyl chloride (0.19 ml) (2.60 mmol) in trimethyl phosphate (2.5 ml). The pale yellow solution was stirred at room temperature for 16 h and then poured onto ice water (25 ml). The water solution was neutralised with Bio-Rad AG-1-X-2 (100-200 mesh) OH^- resin, the resin was filtered off and washed with water-methanol (1:1). The solutions were combined and concentrated under reduced pressure. The residue was co-evaporated with pyridine (3 x 15 ml) and toluene (3 x 15 ml) and was applied to a short column of silica gel which was eluted with chloroform-ethanol (93:7) for (25) and (9:1) for (26). Appropriate fractions were combined and

evaporated to give (25) and (26) respectively. The compounds were crystallised from suitable solvents. (Tables 1 and 2).

Condensation of 5'-chloro-3',5'-dideoxynucleosides (25) and (26) with L-homocysteine sodium salt. General procedure. L-Homocystine (0.268 g, 1 mmol) and sodium (0.092 g, 4 mmol) were added portionwise to a stirred liquid ammonia (25 ml). The blue colour of the resulting solution was discharged with a small amount of ammonium chloride and the 5'chloro-3',5'-dideoxynucleoside (25) or (26) (1 mmol) was added fairly rapidly. The stirring was continued until ammonia evaporated (4-5 h) and the flask with the solid residue was heated in a bath of 40-50°C and evacuated with a water aspirator to remove the residual ammonia. After about 15 min water (50 ml) was added and the solution stirred for about 5 min. Insoluble particles were filtered off and the filtrate was acidified to pH=6.5 with 2M hydrochloric acid and concentrated under reduced pressure. The residue was applied to a column of Sephadex A-25 (2.5 x 20 cm) which was eluted with 0.01M triethylammonium hydrogen carbonate buffer. The appropriate fractions (uv absorbing, ninhydrin positive) were combined concentrated and lyophilised to give (23) (42%) and (24) (51%) respectively. (Tables 1 and 2).

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